# Testing Candidate DNA Quantitation Standards with Real-Time Quantitative PCR methods

The Human Identity Project at NIST has been involved with the forensic DNA measurement community since 1990. Over the subsequent years, members of this community have often requested that NIST provide a DNA quantitation standard. Although we have been exploring production and delivery methods for some time via NIST-sponsored interlaboratory challenge studies, until recently, we were unable to establish production and certification protocols that could produce an SRM fit for this purpose.

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In DNA Quantitation Study 2004 (QS04), laboratories evaluated eight different samples using Quantitative Polymerase Chain Reaction (qPCR) methods. Figure 1 displays composite results from this study that suggest qPCR methods differ both with regard to precision and bias. However, it is unclear from these data whether the observed differences are inherent to the methods or reflect differences in the standards used in their calibration.
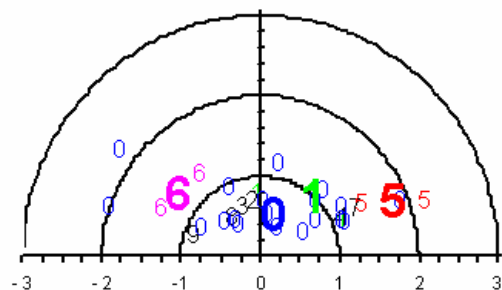

Comparing results from 8 different samples using 10 different qPCR methods, this "Target" plot summarizes among-laboratory measurement performance characteristics. Each small symbol represents a single set ([DNA] of eight samples) of quantitative among-laboratory results: concordance is displayed along the horizontal axis, apparent precision along the vertical axis, and total comparability is the distance from the target center. Method codes are: 0 = Quantifiler, 1 = Alu Q-PCR, 2 = Alu_Sifis, 3 = Alu_tqman, 4 = Aluprobe, 5 = BRCA 1, 6 = CFS-HUMRT, 7 = GB:L78833.1, 8 = RB1, 9 = RTALU. The large bold-face symbols represent the median performance of the among-laboratory results for methods reported by two or more laboratories. Three reference semi-circles are displayed: the inner-most semi-circle delimits a total comparability of one standard deviation from perfect agreement with the consensus medians for all samples, the middle delimits two standard deviations, and the outer delimits three standard deviations.

In this study we evaluated five different qPCR methods using six different human DNA calibration materials. Quantifiler Human DNA Quantification Kit (Applied Biosystems), Quantifiler Y Human Male Quantification Kit (Applied Biosystems), an Alu-based assay, the Centre of Forensic Sciences (CFS) assay, and the California Department of Justice (CA DOJ) assay. Three human genomic DNA standards were obtained from two commercial suppliers (Applied Biosystems, Foster City, CA and Promega Corp., Madison, WI). The DNA concentration ([DNA]) of these commercial materials was used as assigned by their vendor. Three single-source samples were purified at NIST from freshly obtained blood collected in EDTA blood tubes. The extracted samples were analyzed by UV absorbance, scanned from 320 nm to 230 nm. The absorbance at 260 nm was used to assign the DNA concentration in ng/μL for the extracted samples based on the absorbance of 1 being equivalent to 50 ng/μL of double-stranded DNA in Tris-EDTA (TE) buffer (10 mmol/L Tris adjusted to pH 7.0 or 8.0 with HCl + 1 mmol/L EDTA). We designate these standards as "S1" to "S6".

Figure 2 displays the observed crossing threshold (C_Ts) of the different samples at a single concentration of DNA in the Quantifiler Human assay. The C_Ts of samples S2 and S6 differ by 1.1, or a factor of 2.2. There is also a grouping of four samples whose C_Ts are within 0.5 of each other, or within a factor of 1.4. All of the qPCR methods showed a trend, with S3, S4, S5, and S6 being more similar than S1 and S2.
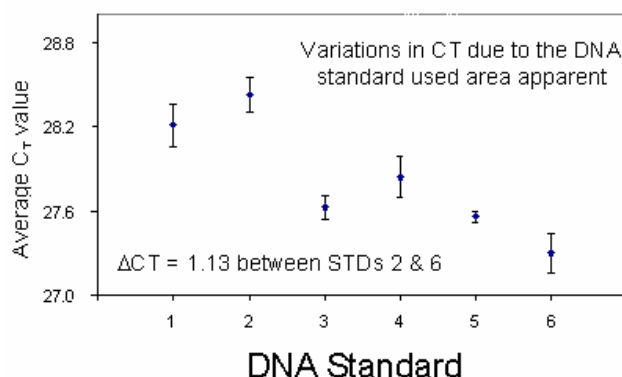
The observed crossing threshold (C_Ts) of the different samples at a single [DNA] in the Quantifiler Human assay. The C_Ts of samples S2 and S6 differ by 1.1, or a factor of 2.2. There is also a grouping of four samples whose C_Ts are within 0.5 of each other, or within a factor of 1.4. Error bars represent ± 1 SD.

There appear to be slight differences in relative sample performance that are consistent among the methods. The qPCR methods appear to yield similar results when the "same" standards are used. The bias seen in QS04 appears to be due to differences in the standards used in their calibration. In essence, we have established that a standard should reduce the bias seen in the different qPCR methods. This is important because laboratories working with new methodologies such as qPCR typically run their own internal validation studies to establish the "ng" quantity that produces a PCR product that generates an acceptable range of signal with their instrumentation. The results from this study may assist those laboratories still "looking" at qPCR methods to realize that the material used to calibrate their system is important.

Based on the results of these studies, NIST is working on the production of a Human DNA Quantitation Standard (SRM 2372).

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Publications:

Kline, M. C., Duewer, D. L., Redman, J. W., Butler, J. M. *Results from the NIST 2004 DNA Quantitation Study*. *J. Forensic Sci.* (2005) 50,,571-578.

Kline, M. C., Duewer, D. L., Redman, J. W., Butler, J. M. *NIST Mixed Stain Study #3: DNA Quantification Practice and its Influence on Short Tandem Repeat Multiplex Performance.* *Anal. Chem.* (2003) 75, 2463-2469.

Duewer, D. L., Kline, M. C., Redman, J. W., Newall, P. J., Reeder, D.J. *NIST Mixed Stain Studies #1 and #2: Interlaboratory Comparison of DNA Quantification Practice and Short Tandem Repeat Multiplex Performance with Multiple-Source Samples.* *J. Forensic Sci.* (2001) 46,1199-1210.

Kline, M.C., Vallone, P. M., Decker, A. E., Redman, J. W., Duewer, D. L., Butler, J. M. *Testing candidate DNA quantitation standards with several real-time quantitative PCR methods.* Proceedings of the 16th International Symposium on Human Identification, online publications at:

http://www.promega.com/geneticidproc/ussymp16proc/abstracts/kline.pdf